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Health and environmental benefits of phytochemicals and antibacterial effectiveness of *Cola nitida* seed extracts on *Salmonella typhi* and *Escherichia coli*

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Abstract

Finding the phytochemical components of *Cola nitida* seeds and assessing the extracts' antibacterial properties against *Salmonella typhi* and *E. coli* were the main objectives of the current investigation. The bacteria (*Salmonella typhi* and *E. coli*) were taken from Usmanu Danfodiyo University's Teaching Hospital in Sokoto and were confirmed by a biochemical test. The extract of bitter kola (*Cola nitida*) was gathered from the Sokoto Old Market, Sokoto South Local Government, Sokoto. the flavonoids, tannins, glycosides, alkaloids, cardiac glycosides, steroid, volatile oil, balsam, and saponin glycosides that make up phytochemicals. The Agar well diffusion method was utilised to assess the test bacteria's sensitivity to the extracts. The concentrations of minimum bactericidal concentration (MBC) and minimum inhibition concentration (MIC) were ascertained. The result of this study has shown that the extract of Bitter kola (*Cola nitida*) contained phytochemical components. Components at high concentration include glycosides, alkaloids and volatile oil. While the components observed at moderate concentration includes tannins, flavonoids, saponins, steroids and balsams. The trace/low concentration component is cardiac glycosides. The result of the antibacterial activity has shown that methanolic extracts of *Cola nitida* seeds had a range zone of inhibition from 27.0mm to 19.5 mm at 150 mg/l -50mg/l concentration against *Salmonella typhi*, which shows a great zone of inhibition very close to the control antibiotics, while *E. coli* had inhibition zone from 19.0 -12.0 mg/l at 150 – 50 mg/l which shows moderate inhibition zone. All the tested strain shows a definite MIC and MBC activity which ranges from 8.5 to 3.5 mg/ml. The result confirmed the antibacterial activity of *Cola nitida*

Keywords: *Cola nitida*; Antibacterial; Phytochemical; Bitter kola; Glycosides; Alkaloids and volatile oil

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1. Introduction

Due to their many uses, chemicals derived from plants have recently attracted a lot of attention (Ncube et al., 2008). The most abundant source of pharmaceutical intermediates, current medications, folk remedies, food supplements, and chemical components for synthesized drugs is medicinal plants (Ncube et al., 2008).

Plants have long been recognized as an important source of natural compounds that support human health. Medicinal plants are the best source of a wide range of medications, according to WHO (2006). Nowadays, traditional natural remedies are used by over 80% of people in developed nations (WHO, 2006). Kola nut is a native stimulant which commonly consumed in many West African communities. It is frequently used ceremonially and to show respect for visitors. The original range of *Cola nitida* was from Sierra Leone to the Republic of Benin on Africa's west coast; the forests of Ghana and Côte d'Ivoire had the highest frequency and variability of the species (Opeke, 2002).

According to Chevalier and Perrott (1991), *C. nitida* cultivation began to spread eastward via Nigeria in 1900, reaching Cameroon and the Congo, and then westward to as far as Senegal (Opeke, 2002). *C. nitida* is planted in Senegal, Guinea, Liberia, and Côte d Ivoire, and Ghana towards the western section of Nigeria (Voelcker, 2005). In traditional medicine, *Cola nitida* has been employed as an appetite suppressant and aphrodisiac. According to Esimone et al. (2007), it is also used for the treatment of indigestion, migraine headaches, and morning sickness. It has been directly administered to the skin to treat inflammation and wounds (Newall et al., 1996). Additionally, it is being utilized for gum and tooth cleaning (Esimone et al., 2007).

Cola nitida has been used to decrease vomiting in pregnant women, in addition, it is utilised as a major stimulant to keep alert and withstand weariness by students, truckers and other menial jobs (Chukwu et al., 2006). Because *Cola nitida* contains tannin in addition to caffeine, people who have stomach ulcers should avoid drinking it (Newall et al., 2006; Farook et al., 2011).

The issue of strains being resistant to the majority of synthetic antibiotics is intimately related to the demand for novel antimicrobial medicines. Strong antifungal and antibiotic treatments do not always work against resistant or multi-resistant microorganisms, necessitating ongoing research and development of new medications. Thus, it is imperative that the hunt for novel sources of antibiotics be an ongoing endeavour. Although *Cola nitida* is known to have antibacterial action against bacterial infections, most of the ones employed in the study area are supported by little data.

The enormous diversity of plants found in Africa contributes to the acceptance of conventional medicine and the vast range of healing concoctions used by traditional healers. There is a pressing need to conduct a scientific inventory of the medicinal plants that are used by traditional healers, given that at least 85% of Africans, regardless of their sociocultural background, turn to traditional treatments to treat their illnesses and lessen their suffering. This applies to both urban and rural areas. This is especially true given the difficulty in treating drug-resistant infections using synthetic and conventional therapy. The findings of this investigation will serve as baseline data for future studies and support the antibacterial activity of bitter kola extracts against a few bacterial strains.

The main aim of this research is to examine the phytochemical components of *Cola nitida* seeds and ascertain the antibacterial properties of its extracts against *Salmonella typhi* and *E. coli*.

The study's particular objectives are as follows:

- To use methanol extracts to extract bitter kola
- to identify the phytochemical components found in *Cola nitida* seed methanolic preparations.
- To ascertain whether extracts from *Cola nitida* seeds are susceptible to antibacterial agents
- To ascertain *Cola nitida* seed extracts' Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

The genus Cola, belonging to the *Sterculiaceae* family and found in tropical Africa, has approximately 125 species. Even while some species of cola grow up to 25 metres in height, most are small to medium sized trees. Tropical nations, particularly those in Africa, are home to a multitude of commonly farmed species. With the latter two having the highest economic significance, the most often utilised are *C. verticillata* (Thonn.) *Stapf, C. acuminata* (Pal. de Beauv.) *Schott* and *Endl*., and *C. nitida* (Vent.) Schott and Endl (Lovejoy, 2000).

Perianths can be coloured or white in the blooms of *C. acuminata* and *C. nitida*. Generally, there are two kinds of bare trees: hermaphrodites with either one or two rings of anthers at the bottom of the superior ovary, or males with anthers fused into a single column. Following fertilisation, the ovary separates into individual fruiting carpels, or follicles, which range in number from five to ten. Some species of Cola, including as *C. nitida* and *C. acuminata*, have edible nuts, however the majority of the species yield hard, inedible seed. Certain species of Cola, like *C. acuminata*, are polycotyledonous. The edible species' seeds vary in size up to 5 cm in length and 3 cm in width. They are ovoid, ellipsoid, or angular by compression. The cotyledons, to which the tiny embryo is attached, make up the majority of the seed. When *C. acuminata* has three or four cotyledons—sometimes as many as six—the seed divides into an equal number of pieces, whereas *C. nitida* seeds easily split in half due to the presence of two cotyledons (Irvine, 1996; Keay, 2000; Russell, 2001).

Long before European explorers arrived, kola nuts were commonly used throughout West and Central Africa (Russell, 2001). When Leo Africanus travelled to western Sudan in 1556, he came upon a bitter nut known as "Goro." In Nigeria, kola is referred to by this name. However, Portuguese explorer Edouado Lopez wrote the first accurate account of kola nuts when he reported seeing seeds having 4 cotyledons in 1593 (Chevalier and Perrot, 1911). A report of the specimens that Palisot de Beauvois had collected during his voyage to regions of modern-day Nigeria in 1786 was published in 1805. Russell (2001) identified the local kola tree as *Sterculia acuminata*, one of the species he described.

Ventenat identified a species he received from Mauritius as *Sterculia nitida* that same year. After Schott and Endlicher classified the genus Cola in 1932, both species were included in it. Russell (2001) claims that a deluge of newly named species, each supported by scant evidence, left the structure of kola species in a state of "indescribable confusion" at the start of the 20th century. Clarity wasn't restored until the taxonomic account published in 1911 by French botanists Auguste Chevalier and Emile Perrot. Chevalier designated the five edible kola nut species as *C. nitida* (trade-related), *C. acuminata* (socio-cultural values), *C. ballayi, C. verticillata*, and *C. sphaerocarpa* under the subgenus *Eucola*. Although the latter three species are unknown to be cultivated, when commercial species produce is in short supply, its seeds are occasionally utilised to contaminate it.

Cola species grow in hot, tropical lowland forests that have temperatures between 23°C and 28°C and rainfall that lasts for at least eight months (Ekanade, 2009). Additionally, certain species have been raised in the areas where the forest and savanna merge (Opeke, 2002). Although it has been found as far north as 10º N on the African west coast, it is primarily grows between 6° and 7° north of the equator. The species may survive a minimum of three months of the dry season, although it needs a humid and hot environment with clearly defined wet and dry seasons (Keay et al., 2000).

Furthermore, it is important to remember that *Cola nitida* might be grown in tiny patches far exceeding the drier regions to the north, where development would only be enabled by the presence of moist terrain with a high-water table (Russell, 2001).

There are no continuous kola plantations in the South Eastern region; instead, each homestead has a family residence surrounded by two to fifteen kola trees. Since the kola tree is revered by the Igbo people, it is planted all around the house, where it will constantly converse with the spirits of the ancestors of the people who are buried there. Each compound's backyard is either dotted with plants or planted with them in orchards. Abia, Anambra, Ebonyi, Enugu, and Imo States are the main producing states in the South East zone, with kolanut trading being the main industry (Keay et al., 2000).

The dense population and land tenure structure in the South Eastern region have been identified as contributing factors to the lack of kola plantations in the area. Farmers have become more discouraged even in areas where space is accessible because to the lengthy gestation period of kola trees and the issue of floral incompatibility, which significantly lowers productivity (Keay et al. 2000).

The nuts include caffeine, theobromine and tannin. The nuts are utilised to make cola beverages. Other constituent of these drinks include spice oils, other fragrant compounds (occasionally including the leaves of the tree), caramel for colouring, sweeteners, phosphoric or citric acid, and carbon dioxide to give effervescence (Benjamin et al., 2001).

When chewed, the nuts have historically been employed as a stimulant. It is said to alleviate exhaustion, stop hunger pains, boost brain function, and cut down on the amount of sleep required. Additionally, parts of the plant are utilised in sacrificial rites, marriage ceremonies, child naming ceremonies, village chief inductions, funerals, and other rituals. Traditional medicine also makes use of the leaves, twigs, bark, flowers, and nuts (Sonibare et al., 2009).The nut is utilised to purify water and for colouring. The wood is used for construction, boat building, furniture and joinery, carving, musical instruments and cutlery. It works well as firewood as well. The pods may replace up to 60% of the maize in chicken feed and have been used to create soap and fertiliser (Lim, 2012).

1.1. Phytochemistry

Kola nuts contain high concentrations of xanthine alkaloids, namely kolatin, caffeine (0.6% - 3.0%), and theobromine (up to 0.1%) (Adeyeye et al., 2007). According to Atawodi et al. (2007), the plant has the following composition: 9.73% moisture, 2.72% ash, 3.02% fat, 19.14% protein, 7.3% crude fibre, and 58.09% carbohydrates. Additional xanthine alkaloids, tannins, proanthocyanidins, and anthocyanins, including "kola red," can be obtained from the plant. Compared to Jamaican kola specimens, which yielded 1.93 percent of caffeine, African kola specimens produced 2.24 percent (Mitchell, 2003). The plant generates about 38 percent amino acids (Adeyeye et al., 2007) and considerable quantities of (+)-catechin, (−)-epicatechin, procyanidin B 1 [epicatechin-(4beta-->8)-catechin], and procyanidin B2 [epicatechin-(4beta-->8)-epicatechin] (Atawodi et al., 2007).

Based on research conducted by Atawodi et al. (2007) and Fontenot et al. (2007), as well as Solipuram et al. (2009), several of the active compounds have been determined to be non-steroidal substances that are bioactive against prostate and breast cancer cells. These compounds may be the cause of the observed bioactivity against these cell lines. Phenolics and anthocyanins are also expected to show antioxidant activity. Many toxins are precipitated in the gut by the tannic acid in kola nuts, which stops the poisons from being absorbed (Lowe et al., 2012).

1.2. Antibiotic Resistance Gram Positive Bacteria

1.2.1. Escherichia coli

Following two outbreaks in Oregon and Michigan, this pathogenic strain of *E. coli* was first discovered in 1982 (Riley et al., 1983). Subsequently, the microbe was found in the stool samples of kids suffering from hemolytic uremic syndrome (HUS). Furthermore, from a HUS child's blood that was preserved in 1974, an antibody against this bacterial strain was discovered in 1991 (Law, 2014). Although the identification of this pathogenic *E. coli* strain was not until 1982, it was considered to be responsible for multiple significant epidemic in the US in the 1950s (Law, 2014).

Microbiological Characteristics

Many warm-blooded organisms, or endotherms, have the rod-shaped, gram-negative bacteria *Escherichia coli*, or simply *E.coli*, in their lower intestines. A few serotypes of *E. coli* can cause food poisoning in individuals, but the majority of strains are safe. The beneficial bacteria are a natural component of the gut flora and can produce vitamin K2 and inhibit the growth of pathogenic bacteria by keeping them from colonising the intestine. Growth on Macconkey agar yields deep red colonies because the organism is lactose positive; nevertheless, fermentation of this sugar will lower the medium's pH and cause the medium to darken. On EMB agar, growth results in black colonies with a green-black metallic sheen.

Epidemiology of *E.coli*

In developing nations, contaminated food and water are the main causes of diarrhoea in children, which can result in traveler's diarrhoea. One of the main causes of diarrhoea in children in underdeveloped countries is contaminated food and water, which can spread from person to person and result in a course of sterile diarrhoea. The main sources of *E. coli* are dairy beef cattle, which can carry the infection asymptomatically and excrete it in their faeces (Cheesbrough, 2002).

Classification of *E.coli*

- Domain: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: *Enterobacteriales*
- Family: *Enterobacteriaceae*
- Genus: *Escherichia*
- Species: *E. coli*

1.2.2. Salmonella typhi

S. typhi is a multi-organ pathogen that was first identified by Karl J. Erberth in 1880. It lives in the lympathic tissues of the small intestine, liver, spleen, and bloodstream of people with the infection. Travellers visiting Asia, Latin America, and Africa are at a higher risk due to the fact that it is still unknown to infect animals and is more prevalent in underdeveloped nations with subpar sanitary systems and limited access to antibiotics. About 70% of the 266

Americans who contracted the sickness in 2002 had left the country within six weeks of it starting Health (Canada: "MSDS of Infectious Substances").

Microbiological Characteristics

A member of the Enterobacteriaceae family, this enteric bacillus is gram-negative. The anaerobe is facultative and motile, and it can be affected by several kinds of antibiotics. As of right now, 107 variants of this organism have been identified; many of them have different metabolic traits, virulence levels, and multi-drug resistance genes that make treatment more difficult in regions where resistance is common. The bacteria are totally non-lactose fermenting, and diagnostic identification can be obtained by growing on MacConkey and EMB agars. In TSI medium, it does not create any gas, which is another characteristic that sets it apart from other Enterobacteriaceae (Kuijpers et al., 2017).

Epidemiology

Humans come into contact with *S. typhi* through the fecal-oral pathway, which connects infected people to healthy ones. Eating shellfish from contaminated bodies of water and secondary infections from individuals who have shed the organism due to poor hygiene are two possible outcomes. On the other hand, ingesting water contaminated by the urine and faeces of infected people is the most frequent source of infection. It is estimated that 100,000 bacteria are required for infection in an inoculum. Typhoid fever additionally symbolizes the 2nd most frequently recorded laboratory infection ("Dennis Kunkel Microscopy, Inc") (Karkey et al., 2013).

The most prevalent way for this species of bacteria to enter the human body is through ingestion; the significance of aerosol transmission is uncertain. Following ingestion, the organisms divide for one to three weeks in the small intestine before bursting the intestinal wall and moving on to other organ systems and tissues. Because of their propensity to multiply intracellularly after absorption and their inhibition of oxidative lysis, the innate host defences are not very effective at preventing infection. The sole known method of *S. typhi* transmission is fecal-oral, frequently from asymptomatic patients. Of those who have been previously infected, 2–5% develops into chronic carriers, meaning they actively shed live organisms that can infect others while displaying no symptoms of illness. "Typhoid" Mary Mallon, a food handler who infected at least 78 individuals and killed five, is a well-known example. Because they don't exhibit symptoms associated with the disease, these highly contagious carriers represent a serious threat to public health. If therapy is initiated early in the infection, typhoid fever damage is limited and reversible. According to Dennis Kunkel Microscopy, Inc., this results in a death rate of less than 1% among treated persons who possess a antibiotic-susceptible strain of *S. typhi*, meaning that patients have a positive prognosis and outcome (Karkey et al., 2013).

Classification of *Salmonella typhi*

- Phylum Proteobacteria,
- Class Gamma Proteobacteria,
- Order *Enterobacteriales*
- Family *Enterobacteriaceae*
- (Dennis Kunkel Microscopy, Inc).

2. Materials and Methods

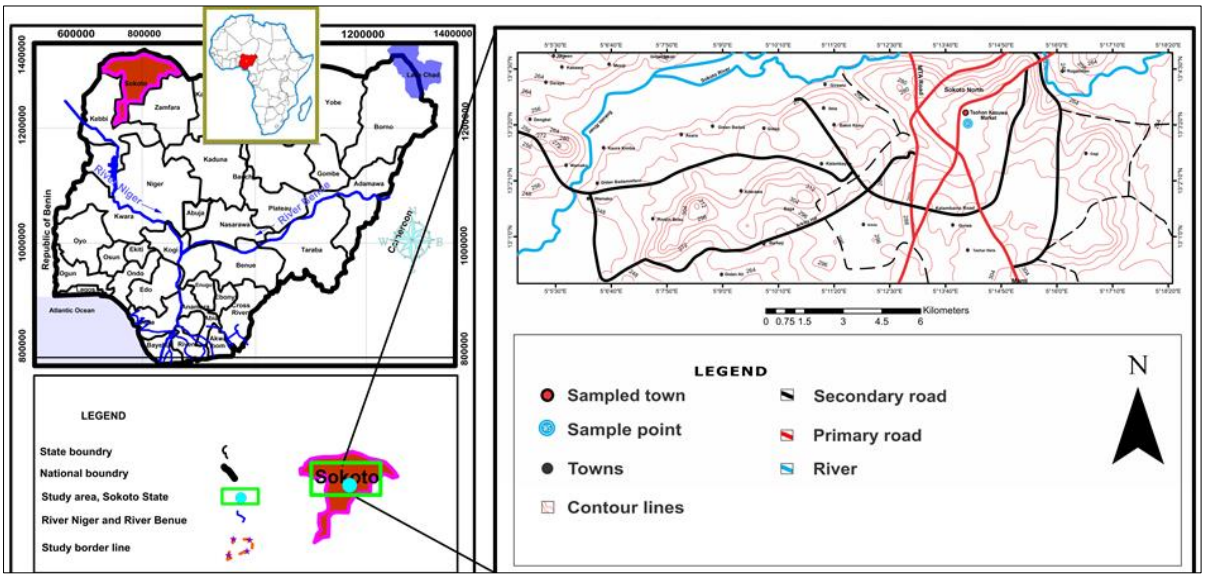

Figure 1 A map of a Study area and its environs with a sample point location. (Modified after Baba Aminu et al., 2023a; Akagbue et al., 2023a; Ibrahim et al., 2023a; Ibrahim et al., 2023b)

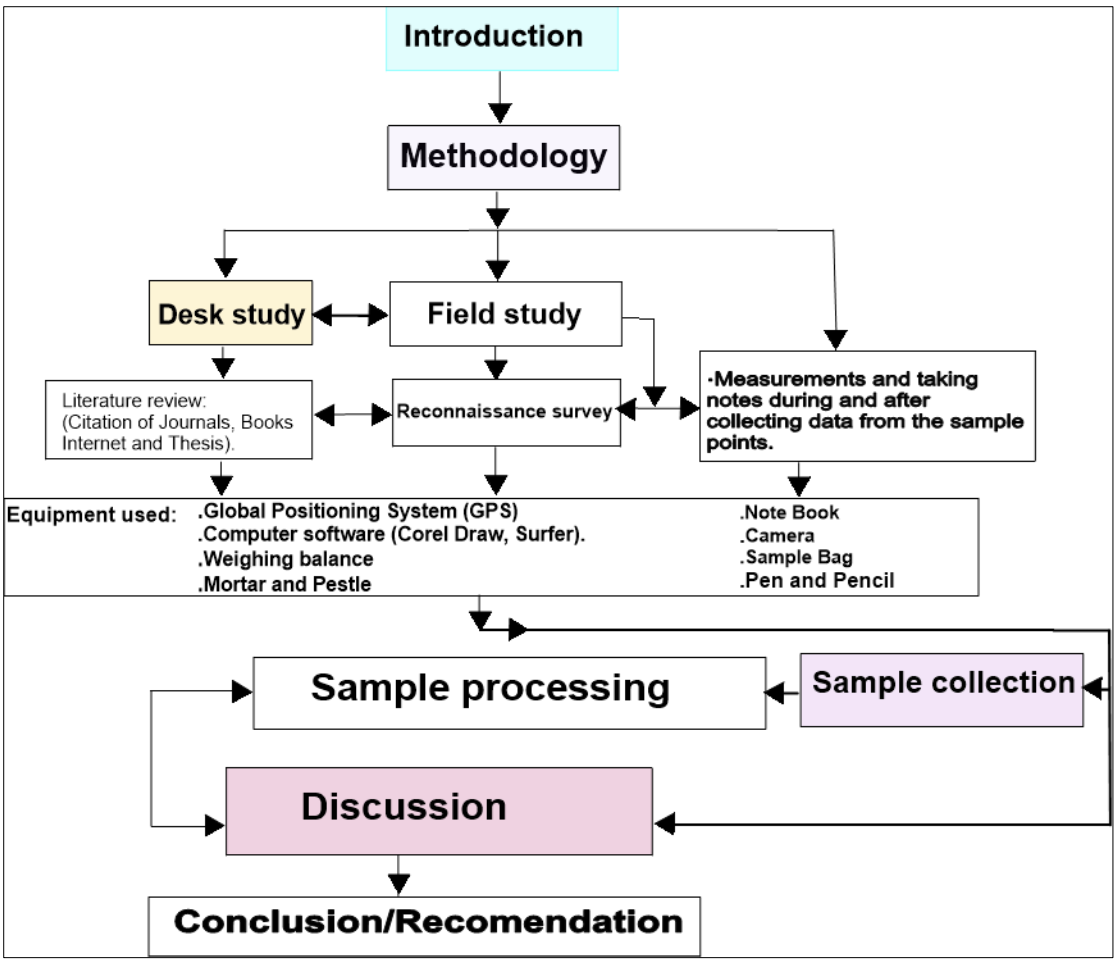

Figure 2 A research flow chat (Modified after Babale et al., 2022)

2.1. Collection of Samples

2.1.1. Plant Materials

The Sokoto Old Market, located in the Sokoto South Local Government, is where the *Cola nitida* seeds were gathered (see fig, 1). It was located in the herbarium of Usmanu Danfodiyo University in Sokoto, where the voucher number (UDUH/ANS/0124) was also placed.

2.1.2. Test Organisms

Standardized isolates of *E.coli* and *Salmonella typhi* were obtained at Usmanu Danfodiyo University, Teaching Hospital Sokoto for evaluation. A sterile Petri dish carrying nutritional agar was injected with the isolates of every test organism that was chosen for analysis.

2.2. Sample Processing

The seeds were cleaned using tap water, then chopped into pieces and allowed to air dry in the shade for a week. After that, they were ground into a powder using a mortar and pestle at the Usmanu Danfodiyo University's Mycology Laboratory in Sokoto. After that, it was sent to Usmanu Danfodiyo University's Microbiology Laboratory for additional examination.

2.3. Media Preparation

2.3.1. Nutrient Broth

The nutrient broth medium was made in accordance with the manufacturer's instructions, weighing 25 g per 1000 ml. 80ml of sterilised distilled water was used to dissolve 2g of nutritional broth, which was then autoclaved for 15 minutes at 121^{o}C to sterilise it (Cheesbrough, 2002).

2.3.2. Muller Hinton agar

The manufacturer's instructions for preparing Muller-Hinton agar (21.5g in 1000ml) were followed.144 millilitres of sterile distilled water were used to dissolve 3.09 grammes of Muller Hinton agar. The mixture was then homogenised and sterilised in an autoclave for 15 minutes at 121 degrees Celsius (Cheesbrough, 2002).

2.4. Extraction Procedure of *Cola nitida* seeds

The seed extract will be obtained by following Aishmma and Mitscher's (2000) methodology. Methanol was used to extract the active component from each of the bitter kola powdered particle. 95 percent methanol was added to 100 grammes (100g) of ground materials, and the mixture was left on its own for 72 hours. Using sterile Whatman's No. 1 filter paper, each extract was separatedly filtered, and filtrates were dried by evaporating them before being kept in a deep freezer for later use.

2.5. Phytochemical Screening

The secondary metabolites found in *Cola nitida* seeds were identified through phytochemical research. The phytochemical test that was conducted out include alkaloids, saponins, tannins, flavonoids, cardiac glycoside, carbohydrates and phenol as stated by Trease and Evans, (2002).

2.5.1. Test for Alkaloids

On a steam bath, approximately 0.5g of the C. nitidas seed extract was dissolved in 5 mL of 1% HCl separately. A small amount of Meyer's reagent was added to one millilitre of the filtrate. Additionally, Wagner's reagent was added to 1 mL of filtrate once more. Reagent-induced turbidity or precipitations were interpreted as proof of the existence of alkaloids (Trease and Evans, 2002).

2.5.2. Test for Saponins (Frothing test)

Each plant extract was diluted with twice as much water (around 2 ml) and violently shaken in a test tube for forty-five minutes. It was then given a minute to stand on the bench. The presence of saponins is indicated by the formation of honeycomb froths that persist for fifteen minutes (Trease and Evans, 2002).

2.5.3. Test for Flavonoids

The extracts were diluted in two millilitres of distilled water and boiled for Shinoda's test. Four drops of strong hydrochloric acid were then added, and finally, some magnesium chips were added. Orange coloration indicates flavones immediately, red crimson coloration indicates flavonoids, and pink magenta coloration indicates flavonoids. A few drops of ferric chloride solution was included after diluting 2 millilitres of the extract with distilled water to a ratio of 1:4. According to Trease and Evans (2002), the occurrence of phenolic nucleus is indicated by a green or blue hue.

2.5.4. Test for Tannins

A clean test tube was filled with one millilitre of the filtrate, and a few drops of ferric chloride were put in for the ferric chloride test. Tannins are indicated by the emergence of blue-black hue. To conduct the lead sub-acetate test, one millilitre of the extract was combined with a few drops of lead-sub-acetate solution. A coloured precipitate suggests the occurrence of tannins (Trease and Evans, 2002).

2.5.5. Test for Cardiac Glycosides

Chloroform was used to extract the extracts, and when the chloroform layer dried completely, it was dissolved in 2 millilitres of glacial acetic acid with a drop of ferric chloride solution. The mixture was then put into a clean test tube with 1 millilitre of conc H2SO4 at the test tube's side. A brown ring found at the interfaces that denotes the presence of cardenolides'-deoxy sugar (Trease and Evans, 2002).

2.5.6. Test for Steroids

Two millilitres of acetic anhydride were added to five grammes of ethanolic extract and two millilitres of sulfuric acid. Colour change from violet to blue or green suggests the presence of steroids (Trease and Evans, 2002).

2.5.7. Volatile Oil

Using dil. HCl, one millilitre of the fraction was combined in this way. The formation of a white precipitate suggested evidence of volatile oils (Trease and Evans, 2002).

2.5.8. Saponin Glycosides

2.5 millilitres of Fehling's solutions A and B were added to 2.5 millilitres of the extract in this procedure. Saponin glycosides were present as a bluish green precipitate (Trease and Evans, 2002).

2.5.9. Anthraquinone

In this procedure, the extract was placed in a test tube, two millilitres of 10% hydrochloric acid were added, and the mixture was heated for roughly two minutes. Equal amount of chloroform was introduced to the test tube and vortexes twice, the layer of chloroform was pipette out and then a similar quantity of 10% ammonia was added. According to Trease and Evans (2002), anthraquinones were present in the upper layer due to a pinkish red hue.

2.6. Determination of Antibacterial Activity of *Cola nitida* seeds

Agar-well diffusion, as described by Akinpelu (1999), was used to screen the crude extracts for antimicrobial activity. Muller-Hinton agar served as the medium. 18 millilitres of sterile, melted Muller-Hinton agar medium that had been cooled to 45 0C was combined with a loop containing the test organism's standardised broth culture, using an inoculating loop to enable the process. After mixing and adding to sterile petridishes, this was put aside to solidify. The needed numbers of holes were drilled into the medium using 6 mm sterile cork borer. With a notation indicating this, the wells were made approximately 5 mm from the plate's edge. Once the proper antimicrobial solutions were made from the medium, each well would fill up. Before being properly diffused into the medium for twenty-four hours at 37 0C, the plates were let to stand on the bench for an hour. Measured and documented in millimetres was the organism's relative susceptibility to the extract, as demonstrated by distinct growth inhibition zones surrounding the wells. A sterilised, melted Muller-Hinton agar medium was equally swabbed with the test organisms. The antibiotic under study was tetracycline.

2.6.1. Determination of Minimum Inhibitory Concentration of the Extract

Using the technique outlined by Akinpelu and Kolawole (2004), the MICs of the extract (methanol, aqueous, and n-hexane) will be determined. A range of concentrations between 150, 100, and 50 mg/ml were achieved by adding 2 ml of varying concentrations of the C. nitida seed extract solution to 18 ml of pre-sterilized molten nutritional agar in a

McCartney bottle at 45 0C. This was done after preparing two-fold dilutions of the extract. After being added to sterile Petri dishes, the media was left to set. Before streaking the nutrient agar plate with a typical 18-hour-old broth culture of the sensitive bacteria, the surface was allowed to dry. After properly labelling each plate and incubating it for 24 hours at 37 0C, the test organisms were checked to see if they were growing or not. The Minimum Inhibitory Concentrations (MICs) of the extract were determined by taking the lowest concentration at which the test organisms could not grow.

2.6.2. Determination of Minimum Bactericidal Concentrations (MBC)

Using the methodology outlined by Ngoupayo *et al*. (2015), the MBCs of the methanolic extract were evaluated. After being sub-cultured onto recently made, sterile nutrient agar plates, samples from plates in the MIC assay plate that showed no visible growth were incubated at 37 ^{0}C for a full day. The minimal amount of extract that was bactericidal was determined by taking the smallest concentrations of the extract that did not cause any growth on a fresh set of nutrient plates.

3. Results

Table 1 displays the phytochemical contents analysed in *Cola nitida* seeds, including tannins, flavonoids, saponis, alkaloids, cardiac glycoside, saponin, saponin glycoside, sterpids, balms, antharquinone and volatile oil. High concentrations were identified for glycosides, alkaloids, and volatile oil; moderate concentrations were found for flavonoids, tannins, saponin, steroids, and balsam; trace to low concentrations was found for cardiac glycosides; no saponin glycosides were observed.

Cola nitida methanol extracts have antibacterial action against *E. coli* and *Salmonella typhi*, as can be seen in Table 2. It has observed that *Salmonella typhi* had a zone of inhbition varied from 27.0mm to 19.5mm at 150mg/l to 50mg/l concentration. At 150 mg/l to 50 mg/l, the *E. coli's* inhibition zone ranged from 19.0 to 12.0 mg/l.

The lowest bactericidal and inhibitory concentrations of the extracts are displayed in Table 3. It demonstrates that the observed minimum inhibitory concentration against the strain under test is greater than the minimum bactericidal concentration.

Table 1 Phytochemical Screening of the *Cola nitida* Seed Methanol Extracts

Phytochemicals	**Results**
Flavonoid	++
Tannins	++
Saponins	++
Glycosides	+++
Alkaloids	+++
Cardiac glycosides	+
Steroid	++
Balsams	++
Volatile oil	+++
Saponin Glycosides	ND

Key: ND = Not detected, + = Trace, ++ = Phytochemical moderately; present ,+++ = = phytochemicals present in high concentration.

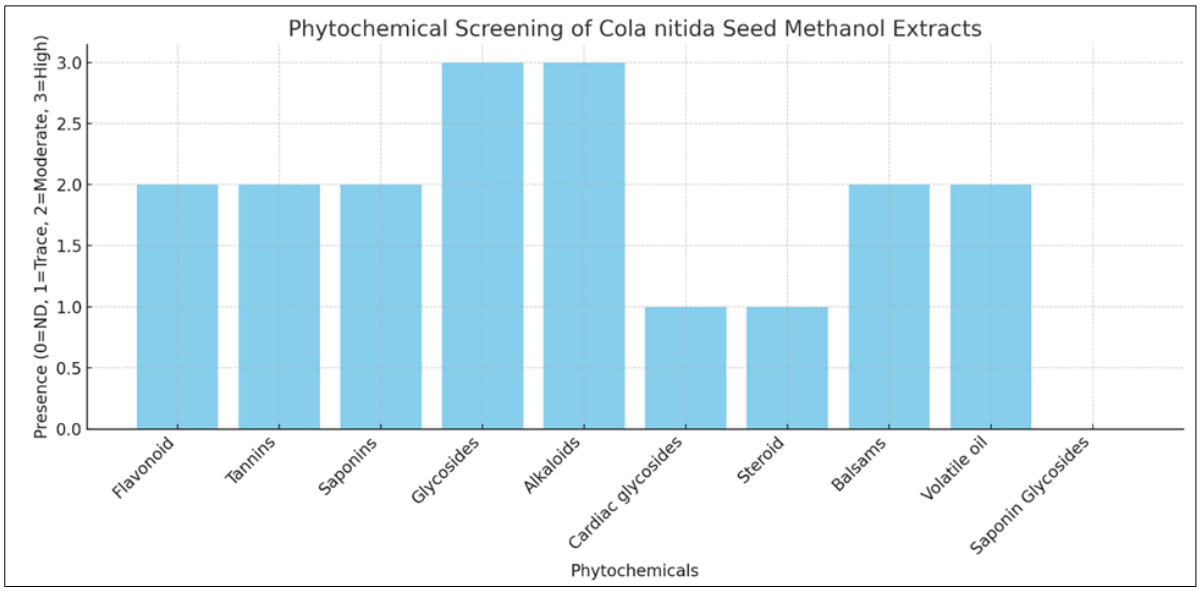

Figure 3 Phytochemical Screening of *Cola nitida* Seed Methanol Extracts (Table 1)

The bar graph shows the presence of different phytochemicals in the *Cola nitida* seed methanol extracts. A score of 0 indicates 'Not Detected' (ND), 1 indicates a 'Trace', 2 indicates 'Moderate presence', and 3 indicates 'High concentration'. Glycosides and Alkaloids are present in high concentrations, while Cardiac glycosides, Steroid, and Saponin Glycosides are either present in trace amounts or not detected.

Table 2 Antibacterial Activity of *Cola nitida* Methanol Extract against Tested Bacteria

Bacteria	**Zone of inhibition (mm)**			**Control**
	150mg/ml	**100mg/ml**	**50mg/ml**	
Salmonella typhi	27.0	23.0	19.5	27
Escherichia coli	19.0	16.5	12.0	30

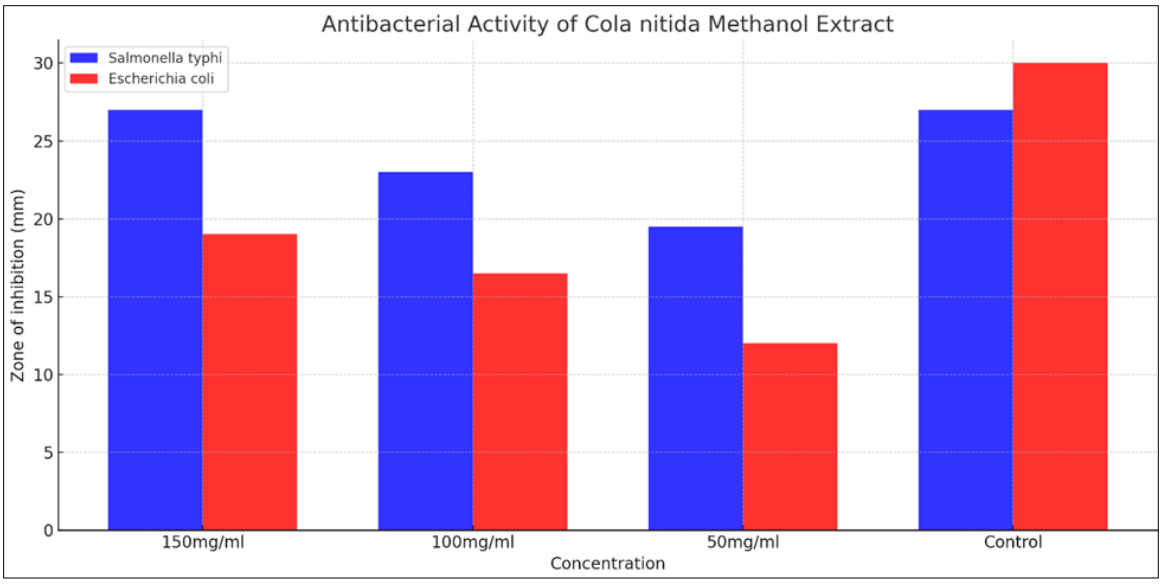

Figure 4 Antibacterial Activity of *Cola nitida* Methanol Extract (Table 2)

This graph plots the zone of inhibition in millimeters against different concentrations of the *Cola nitida* methanol extract for two bacteria: *Salmonella typhi* and *Escherichia coli*. The zone of inhibition decreases as the concentration decreases for both bacteria, indicating a dose-dependent antibacterial effect. The 'Control' shows the zone of inhibition without the extract, providing a reference for the maximum inhibition observed.

Table 3 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Methanol Extracts

Isolates	MIC(mg/ml)	MBC(mg/ml)
Salmonella typhi	4.4	4.0
Escherichia coli	8.5	3.5

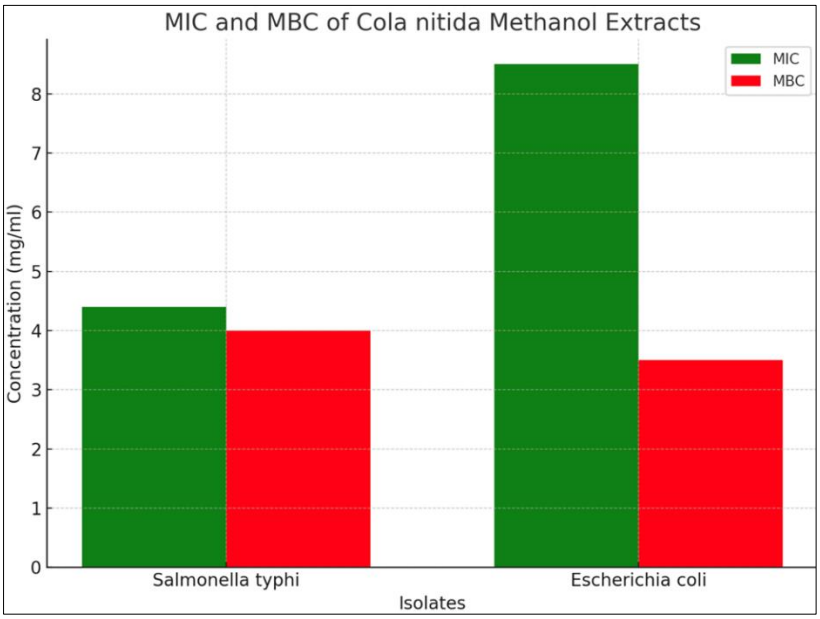

Figure 5 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Methanol Extracts (Table 3)

The bar graph displays the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) for each isolate. The MIC is the lowest concentration that prevents visible growth of a bacterium, while MBC is the lowest concentration that results in microbial death. The graph shows that *Salmonella typhi* has a lower MIC and MBC compared to *Escherichia coli*, suggesting that it is more susceptible to the *Cola nitida* methanol extract.

The findings suggest that the *Cola nitida* methanol extract contains various phytochemicals with significant antibacterial properties, with varying effectiveness against the tested bacteria.

4. Discussion

Medicinal herbs constitute a rich supply of antibacterial compounds. Worldwide, plants are utilised medicinally and are a good source of many strong and potent medications. Even before the development of artificial antibiotics and other contemporary medications, the use of plant-derived treatments in traditional medical practices was prevalent and common in Africa (Rukangira, 2012). (Olajuyigbe and Afolayan, 2012). A search for new antimicrobial drugs from alternative sources, such as plants, which are good raw materials for new antimicrobial chemotherapeutic agents, has become necessary due to issues with microbial drug resistance, the rise in opportunistic infections, and the toxicity effect of continued use of multiple antimicrobial agents (Sasidharan et al., 2011).

The antibacterial properties of *Cola nitida* extracts against *Salmonella typhi* and *E. coli* were investigated, as well as their phytochemical composition. The study's findings showed that bioactive substances found in *Cola nitida* seeds included antharquinone, tannins, flavonoids, saponin, alkaloids, cardiac glycoside, sterpids, and volatile oil. High concentrations of glycosides, alkaloids, and volatile oil were seen; moderate concentrations of flavonoids, tannins, saponin, steroids,

and balsam were noted; trace or low concentrations of cardiac glycosides were discovered; no saponin glycosides were recognised. Thus, this result is consistent with the Sofowora (1993) findings. It is also in acceptance with Adegboye et al., (2008) and Eminedoki et al.(2010). It is believed that the active elements displaying antibacterial infection opposed to the tested isolates are these group phytochemical substances. These phytochemical substances, which have specific physiological effects on the human body, are the basis of these plants' medical usefulness.

Certain plants, such as those found in humid rain forests, have been demonstrated to possess alkaloids, which confer unique antibacterial properties and possibly provide a competitive advantage over microorganisms (Bringman et al., 1992).

Moreover, red blood cells might precipitate and coagulate when exposed to saponin. According to Sodipo et al. (2000), some of the traits of saponins are their ability to produce bubbles in aqueous solutions, hemolytic activity, cholesterol-binding qualities, and bitterness. Given that kolanuts are widely recognised as a sociable fruit that people of all ages consume, the mild levels of saponin in *Cola nitida* that have been studied may not be harmful to the user (Dah-Nouvlessounon et al., 2015). However, more testing on this toxicity issue is still possible. Numerous hydrolytic enzymes, including the proteolytic macerating enzymes employed by plant species, are effectively inhibited by phenolic substances found in plant cells, such as tannins and flavonoids. Furthermore, non-toxic glycosides found in many plants have the ability to hydrolyze and produce phenolics, which are hazardous to microbial pathogens (Jayalakshmi et al., 2011).The antibacterial action of *Cola nitida* is most likely due to the occurrence of these chemicals. The plants may contain biological properties such as defence against allergies, inflammatory conditions, free radicals, platelet aggregation, bacteria, ulcers, hepatoxins, viruses, and even tumours, according to the comparatively high concentrations of saponins found in the examined *Cola ntitida* preparations (Okwu, 2004).

Cola nitida and *C. acuminata*, respectively, show therapeutic impact on blood diseases, obstetrical diseases, and digestive system disorders, according to a survey done in the Benin Republic by Dah-Nouvlessounon et al. (2015). According to Okwu (2004), flavonoids are powerful antioxidants that are soluble in water and free radical scavengers that shield against various stages of carcinogenesis, inhibit oxidative cell damage, and have substantial anticancer potential.

Salmonella typhi and *E. coli* were tested for antibacterial activity using *Cola nitida* methanol extracts. The results showed that *Cola nitida* extracts had the highest part of limitation against *Salmonella typhi*, measuring 27.0 mm at 150 mg/l, which is more similar to the control antibiotics. It also demonstrated an adequate zone of inhibition at 100 mg/ml and 50 mg/ml, measuring 23.0 mm and 19.5 mm, respectively. On the other hand, the *Escherichia coli* strain, which ranged in size from 19.00 mm to 12.0 mm at 150 mg/ml to 50 mg/l, showed moderate inhibitory efficacy. These bacterial strains' suppression is linked to the bioactive substances found in *Cola nitida* seeds, including tannin, cardiac glycoside, alkaloid, and saponin, among many others. This is consistent with the findings of Ekanade (2019), who found that *Escherichia coli* is among the many bacterial species to which *Cola nitida* is effective. The outcomes also demonstrated that the minimal inhibitory doses exhibited greater inhibitory activity as compared to bactericidal action due to their greater effectiveness. This contrasts with the findings of Adegboye et al. (2008), who found that, when compared to conventional antibiotics, extracts from Cola spp. seeds demonstrated very good bactericidal activity. This may be a result of the various Cola species utilized and the extraction methods.

5. Conclusion

The conclusion is that bioactive components included in *Cola nitida* seeds contribute to their antibacterial qualities. *Cola nitida* seeds have also been shown to have some inhibitory effect on *Escherichia coli* and *Salmonella typhi*.

Recommendations

- To provide a more precise assessment of the plant's medicinal potential, research on *Cola nitida's* antibacterial efficacy against a larger variety of clinical isolates of pathogenic organisms is necessary.
- To evaluate their therapeutic application, it will also be important to clarify the mechanism of action and levels of toxicity.
- To determine the proactive elements included in *Cola nitida* extracts for usage in the future, more research must be done.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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